☐ 1: J Immunol Methods. 1993 Dec 3;166(2):281-5. Related Articles, Links

The role of pH in modified ELISA procedures used for the estimation of functional antibody affinity.

Goldblatt D, van Etten L, van Milligen FJ, Aalberse RC, Turner MW.

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Solid phase assays for the measurement of functional antibody affinity are increasingly being used in both clinical and research settings. The majority of such assays employ a chemical reagent to disturb antibody binding but relatively little is known about the properties of such reagents and the basis of their effect on antigen-antibody binding. We have evaluated the diethylamine (DEA) ELISA procedure for the measurement of functional antibody affinity in two independent assays, one for functional human IgG subclass affinity to an organism, Moraxella catarrhalis, and the other for measuring functional affinity of mouse monoclonals specific for the cat allergen Fel d I. DEA was shown to increase the pH of the buffering solution and it was this rise in pH that affected antibody binding. Alkaline buffer and DEA were equally efficient in the inhibition of binding of both the human IgG subclasses and the two mouse monoclonal antibodies to the solid phase. In contrast, pH was shown to have no role in the chaotropic effect of the ion, thiocyanate.

PMID: 8288881 [PubMed - indexed for MEDLINE]

☐ 1: Nucl Med Biol. 1993 Apr;20(3):311-5. Related Articles, Links

A simple method for affinity purification of radiolabeled monoclonal antibodies.

Juweid M, Sato J, Paik C, Onay-Basaran S, Weinstein JN, Neumann RD.

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A simple method is described for affinity purification of radiolabeled antibodies using glutaraldehyde-fixed tumor target cells. The cell-bound antibody fraction is removed from the cells by an acid wash and then immediately subjected to buffer-exchange chromatography. The method was applied to the D3 murine monoclonal antibody which binds to a 290 kDa antigen on the surface of Line 10 guinea pig carcinoma cells. No alteration in the molecular size profile was detected after acid washing. Purification resulted in a significant increase in immunoreactivity by an average of 14 +/- 47% (SD; range 4-30%).

PMID: 8485490 [PubMed - indexed for MEDLINE]

☐ 1: Cancer Res. 1983 Feb;43(2):686-92.

Immunological heterogeneity of carcinoembryonic antigen: antigenic determinants on carcinoembryonic antigen distinguished by monoclonal antibodies.

Primus FJ, Newell KD, Blue A, Goldenberg DM.

Murine monoclonal antibodies against carcinoembryonic antigen (CEA) derived from a colonic tumor were analyzed by radioimmunoassay for reactivity with CEA and the CEA-related antigens, meconium antigen (MA) and nonspecific cross-reacting antigen. Antibody-antigen binding profiles revealed three classes of hybridomas. The Class I antibody, NP-1, recognized an epitope shared among all three antigens, and its affinity for CEA and MA was high but low for nonspecific cross-reacting antigen. The Class II antibodies reacted with sites shared between CEA and MA, while those of the Class III type only bound CEA. The Class III antibody, NP-4, bound less than 50% of the CEA molecules recognized by goat specific anti-CEA antibody and the other classes of monoclonal antibodies. Two Class II antibodies, NP-2 and NP-3, bound similar amounts of CEA and MA with moderate but different affinities for CEA. Studies using labeled monoclonal antibodies for CEA epitope blocking revealed that NP-2 and NP-3 recognize two separate epitopes on CEA within the Class II category. Thus, monoclonal antibodies to CEA can differentiate at least four antigenic sites on colonic cancer CEA. One is shared by CEA, MA, and nonspecific cross-reacting antigen; two others are shared by CEA with MA; and a fourth appears specific for a subpopulation of CEA molecules.

PMID: 6184152 [PubMed - indexed for MEDLINE]

1: Mol Immunol. 1982 Dec;19(12):1641-8. Related Articles, Links

Specificities and binding properties of eight monoclonal antibodies against carcinoembryonic antigen.

Hedin A, Hammarstrom S, Larsson A.

Eight different monoclonal antibodies against CEA were derived from fusions with spleen cells of mice immunized with highly purified CEA. All eight antibodies were IgG l, kappa and had isoelectric points between pH 6.5 and 7.5. They reacted strongly with native CEA, Smith degraded CEA (SI-stage) and CEAlow, only marginally with reduced and alkylated CEA and not at all with orosomucoid, indicating that they were directed against conformation dependent protein determinants. Two antibodies cross-reacted strongly with nonspecific cross-reacting antigen (NCA) while none of the antibodies cross-reacted with biliary glycoprotein I (BGP I). At least six different epitopes in the peptide moiety of CEA were recognized by this series of monoclonal antibodies. At least two of these appeared to occur twice in the molecule, possibly indicating that CEA contains two or more homology regions. The affinity constants of three of the CEA-'specific' antibodies were found to be very high, e.g. 1.2, 3.3, and 7.4 X 10(8) M-1, respectively.

PMID: 6186908 [PubMed - indexed for MEDLINE]